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Hereditary Desmoid Disease Due to a Frameshift Mutation at Codon 1924 of the *APC* Gene

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Summary

Desmoid tumors are slowly growing fibrous tumors highly resistant to therapy and often fatal. Here, we report *hereditary desmoid disease* (HDD), a novel autosomal dominant trait with 100% penetrance affecting a three-generation kindred. Desmoid tumors are usually a complication of familial adenomatous polyposis, a predisposition to the early development of premalignant adenomatous polyps in the colorectum due to chain-terminating mutations of the *APC* gene. In general, one or more members in ~10% of the FAP families manifest desmoid tumors. Affected individuals from the HDD kindred are characterized by multifocal fibromatosis of the paraspinal muscles, breast, occiput, arms, lower ribs, abdominal wall, and mesentery. Osteomas, epidermal cysts, and other congenital features were also observed. We show that HDD segregates with an unusual germline chain-terminating mutation at the 3′ end of the *APC* gene (codon 1924) with somatic loss of the wild-type allele leading to tumor development.

Introduction

Desmoid tumors are rare neoplasms usually occurring either sporadically or as an extracolonic manifestation of familial adenomatous polyposis (FAP). FAP is an autosomal dominant condition predisposing to the development of multiple colorectal adenomatous polyps during adolescence (Bussey 1975). Desmoid disease (fibromatosis) occurs in ~13% of FAP kindreds, can be multifocal, and is more commonly seen in female than in male patients with FAP (Klemmer et al. 1986; Jones et al. 1987). The majority of desmoid tumors occur in the mesentery of the bowel or in the abdominal wall (Bulow 1987; Jones et al. 1987). In a significant percentage of the cases, desmoids present with symptoms, and, although technically nonmalignant, recurrence after surgical removal is usual and death can result because of invasion of local structures (Jones et al. 1987; Rodriguez-Bigas et al. 1994).

In classic FAP, thousands of colorectal adenomatous arise from adolescence (Bussey 1975). Adenomatous polyps are benign tumors (adenomas), a percentage of which inevitably undergo malignant transformation and subsequently metastasize if the affected segment of the bowel is not removed surgically. FAP affects, on average, 1/8,000 individuals, and, although the colorectal adenomas are the clinical hallmark of the disease, it should be regarded as a multisystem disease, because a number of extracolonic manifestations are now well established as part of the overall picture. These features derive from all three embryonic lineages including the mesoderm—infiltrative fibromatosis of connective tissue (desmoids), osteomas, and dental abnormalities; endoderm—adenomas and adenocarcinomas of the upper gastrointestinal (GI) tract, liver, and endocrine tumors; and ectoderm—epidermoid cysts of the skin and congenital hypertrophies of the retinal pigment epithelium (CHRPEs) (Talbot 1994).

The gene responsible for FAP, the adenomatous polyposis coli (*APC*) gene, has been recently isolated and encodes a large (312-kD) protein presumably involved in the intracellular transmission of cell-adhesion signals (Polakis 1995). Recently, overexpression of its gene product in cultured cells indicated that *APC* might also block cell-cycle progression from the G0/G1 to S phase (Baeg et al. 1995).

Several sequence motifs characterize the *APC* polypeptide: the so-called coiled-coil motifs in the NH_2-terminal region allow *APC* to oligodimerize and presumably to participate in large macromolecular structures (Joslyn et al. 1993; Su et al. 1993*a*); the *catenin binding sites* allow interactions among *APC* and α-, β-, and γ-catenin, a group of proteins known to associate with E-

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cadherin in the formation of epithelial cell-cell contacts (the so-called adherens junctions) (Rubinfeld et al. 1993; Su et al. 1993*b*); a COOH-terminal "basic" amino acid cluster thought to be responsible for the binding of *APC* to microtubuli (Munemitsu et al. 1994; Smith et al. 1994). EB1, a novel protein of unknown function, was also found to bind to the *APC* COOH-terminus (Su et al. 1995). More recently, *APC* was shown to bind to the human homologue of the *Drosophila* discs tumor-suppressor protein (DLG) (Matsumine et al. 1996) and to glycogen synthase kinase 3β (GSK3β) (Rubinfeld et al. 1996).

The isolation of the *APC* gene has also allowed the characterization of a large number of disease-causing mutations (to date, more than 450) in individuals affected by FAP (reviewed in Nagase and Nakamura 1993). The majority of mutations identified to date in the *APC* gene are located in the 5′ half of the gene and result in stable truncated polypeptides of different molecular weights (Smith et al. 1993). The large body of data collected on *APC* mutations has greatly contributed to the establishment of genotype-phenotype correlations between the type and location of the mutation along the *APC* gene and the corresponding phenotypic features, i.e., age at onset and number of intestinal polyps, type and number of extracolonic manifestations. Nevertheless, both inter- and intrafamilial variability in the morbid consequences of specific germ-line mutations have been observed, presumably due to unlinked modifier genes and environmental factors.

Here, we report the first family in which multiple desmoid tumors have been inherited through three generations in the absence of the colonic features of FAP. In this kindred multifocal desmoid tumors occur at sites unusual for FAP-related desmoid disease and with 100% penetrance. We show that a chain-terminating mutation at *APC* codon 1924 is responsible for this condition, for which we propose the name of *hereditary desmoid disease* (HDD).

Material and Methods

(CA)$_n$ PCR Analysis

Polymorphic microsatellite markers flanking the *APC* gene were used for linkage analysis and loss-of-heterozygosity studies. The following markers have been employed: CB26 (van Leeuwen et al. 1991), YN5.64 (Breukel et al. 1991), LNSCA (Spirio et al. 1991), and CA25 (Wijnen et al. 1991). The centromere-telomere order of the markers used is shown in figure 1. DNA samples were obtained from peripheral blood leukocytes from all available family members except for patient I.1, where DNA was extracted from a paraffin-embedded epidermoid inclusion cyst, and patients III.2 and III.6, where additional DNA samples were extracted from tumor tissue. For each of the four primers, one primer of each pair was end labeled by using ^{32}P-γATP and T4 polynucleotide kinase. Genomic DNA (100 ng) and 10 pmol of each primer were used in a total volume of 20 μl containing 10 × PCR buffer (Perkin Elmer), 1.5 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate, and 0.5 U *Taq* polymerase (Perkin Elmer). Samples were heated at 95°C for 6 min followed by 30 cycles (10 s at 94°C, 30 s at 55°C, and 30 s at 72°C) and a final extension period of 7 min at 72°C. PCR products were separated on a 5% polyacrylamide/urea gel and visualized by autoradiography.

Protein-Truncation Test (PTT)

The PTT was performed as described by van der Luijt et al. (1994, 1996). In brief, templates for the PTT analysis of exon 15 were generated by PCR on 100–200 ng DNA by using the T7-modified primers described elsewhere (van der Luijt et al. 1994). PCR was performed in a 50-μl volume containing 10 mM Tris.HCl pH 8.9, 50 mM KCl, 2 mM $MgCl_2$, 200 μg/ml BSA, 0.01% gelatin, 0.2 mM of each dNTP, 10% glycerol, 15 pmol of each primer, and 1 unit of AmpliTaq DNA Polymerase (Perkin Elmer/Roche Molecular Systems). Amplification was performed for 35 cycles (1 min at 94°C, 1.5 min at 55°C, and 3 min at 72°C), followed by a final extension period of 10 min at 72°C. In vitro transcription and translation reactions were performed in a 12.5-μl reaction volume. SDS-PAGE gels were run at 30 mA for ~3.5 h.

Sequence Analysis

Automated Laser Fluorescent DNA sequencing was performed as described by Wijnen et al. (1995). In brief, the DNA fragment displaying a truncated protein band on PTT was analyzed by direct solid-phase sequencing using the streptavidin-coated magnetic beads M280 (Dynal). To minimize the sequencing efforts required to characterize the mutation detected by PTT analysis between codons 1595 and 2344, the size of the truncated protein (42 kD) was used to estimate the position of the stop codon within the exon. The exon 15M primers (according to the nomenclature reported by Groden et al. 1991) were employed as sequencing primers by covalently linking a biotin group to the 5′ end of the B primer and by adding a universal 23-bp M13 sequence (5′-CGACGTTGTAAAACGACGGCCAG-3′) to the 5′ end of the A primer.

Western Analysis

Analysis of the protein lysate from a fibroblast and a lymphoblastoid cell line derived from patient III.2 was performed by Western blot essentially as described by Smith et al. (1993). Two antibodies were employed: AFPN, a polyclonal antibody raised in our laboratory

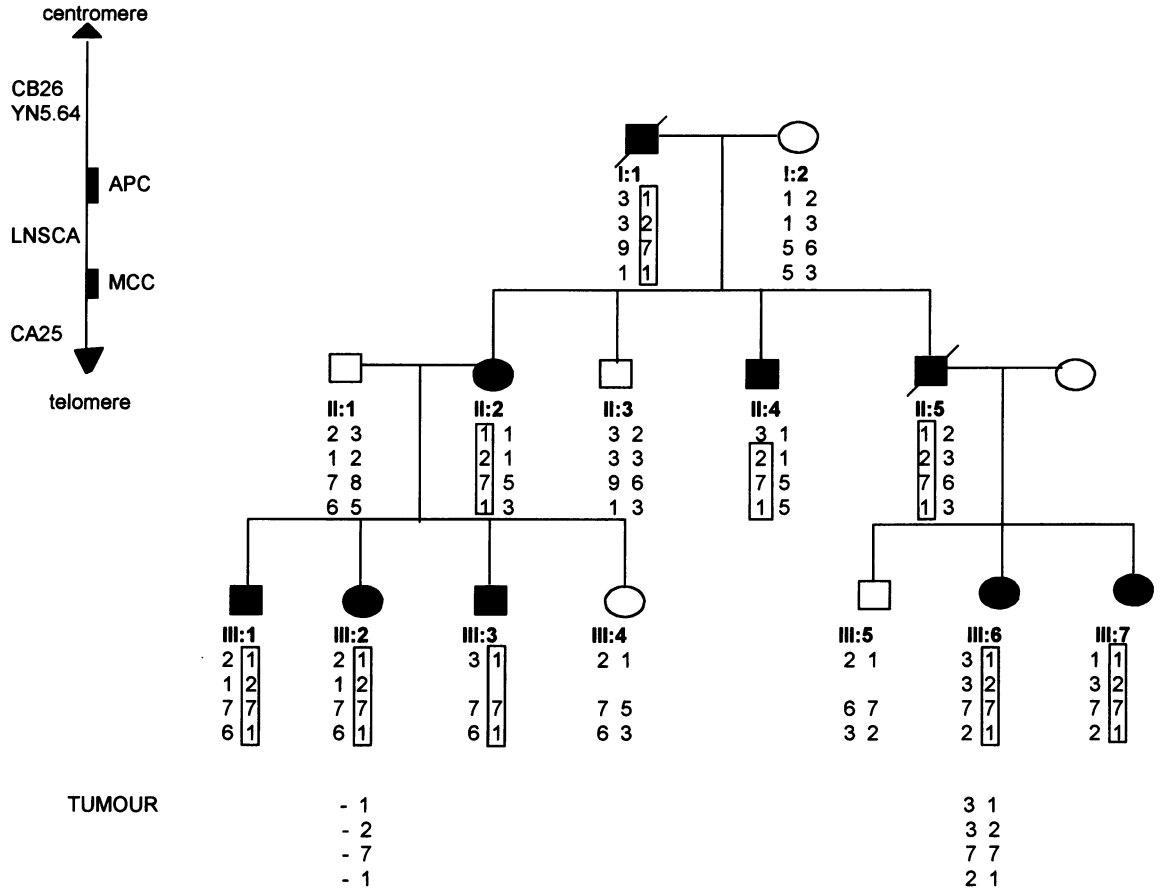

Figure 1 Pedigree structure and results of linkage analysis, including idiogram showing order of polymorphic markers flanking the APC locus. Results of loss of heterozygosity in intraabdominal desmoids from III.2 and III.6 are shown below the relevant patients.

(C.B. and R.F.) against a fusion protein encompassing residues 8–312 of the *APC* protein, and FE9, a commercially available monoclonal (Oncogene Science) raised against amino acids 1–220.

Cytogenetic Analysis

One- to two-centimeter cube portions from each of three separate parts of the intraabdominal desmoid tumor from III.2 were examined. Samples were disaggregated with collagenase 1A (Sigma) for open system culture on cover slips and in situ harvest by the method of Fisher et al. (1996). Growth was rapid and cells were harvested after 3 d and G-banded. Forty-five metaphases were examined from the three samples.

Results

Clinical Findings

The structure and clinical features of the family are summarized, for clarity, in figure 1 and table 1, respectively. An example of desmoid disease in the paraspinal muscles of a family member is shown in figure 2.

The index case (individual III.2 in fig. 1) presented at the age of 15 years with multifocal desmoid tumors in the paraspinal muscles, causing scoliosis. She had noticed an asymptomatic swelling in the lumbar region from early childhood. Surgery was performed, and the largest tumor was resected but regrew over the course of only a few months. Chemotherapy, radiotherapy, and antiestrogen therapy failed to halt the progression of the disease. A colonoscopy was carried out at 26 years of age and showed normal flat mucosa throughout. At 29 years of age, she had a very large intraabdominal desmoid resected involving the superior mesenteric artery and necessitating removal of most of her small bowel and right hemicolon. There was no evidence of colonic polyposis at this time, either macroscopically or microscopically. Her brother (III.1) had similar paraspinal disease arising in the 2d decade; a biopsy confirmed the diagnosis of infiltrative fibromatosis. He was treated with radiotherapy and estrogen antagonists, without response. He did not undergo radical surgery and has had a relatively slow progression of the disease for the past 4 years. At birth, he presented with an imperforate anus. Bowel examination at 30 years revealed no evidence of polyposis. Their grandfather (I.1) died of metastatic

Table 1

Phenotypic Features of Affected Individuals from the Familial Desmoid Disease/FAP Pedigree

Individual (Age in years)	Desmoid Tumors	Polyposis	Other Features
I.1 (died at 62)	2–3-cm firm swelling at nape of neck (recorded in medical notes)—family certain he had several lumps similar to other family members	No investigations; metastatic cancer on admission; palpable rectal cancer	Epidermal cyst removed
II.2 (51)	Bilateral breast desmoid tumors[a]; large desmoid over occiput	<50 adenomatous polyps mainly in transverse and descending colon; gastric fundus polyps also observed	Osteomata of skull and mandible; no CHRPE
II.3 (47)	No skin manifestations; no symptoms	Unaffected; not colonoscoped	None
II.4 (49)	Two small soft-tissue tumors overlying lower rib and arm	No duodenal polyps and a few gastric polyps on upper GI endoscopy (at age 49 years); no colonic polyps at age 49 years	Rarefaction of skull over torcula
II.5 (53)	Several large skin desmoids over arms[a]; paraspinal muscles[a], and occiput	No duodenal polyps on upper GI endoscopy, colonoscopy not done	Died of metastatic carcinoma of the ampulla of Vater
III.1 (30)	Multiple paraspinal desmoid tumors[a]	No polyps on colonoscopy at age 30 years; awaiting gastroscopy	Imperforate anus; osteomas of mandible
III.2 (29)	Multiple desmoid tumors of paraspinal muscles[a], large intraabdominal desmoid[a]	No colonic polyps at age 25 years, no adenomata or microadenomata in colonic resection specimen at age 29 years	Scoliosis due to desmoid disease
III.3 (29)	Two 2-cm small lumps on skull	No colonic polyps at age 31 years, normal upper GI endoscopy	None found
III.6 (31)	Small paraspinal desmoid[a]; large intraabdominal desmoid[a]	No colonic polyps at age 31 years; normal upper GI endoscopy	Epidermal inclusion cyst[a]
III.7 (33)	Small erector spinae desmoid; breast desmoid[a]; on CT scan, abdomen clear	No polyps on colonoscopy; few gastric fundus polyps observed	None found

[a] Histologically confirmed.

cancer at the age of 62 years. He died within 2 wk of hospital admission with jaundice and multiple liver metastases from a clinically palpable rectal carcinoma, although this was never confirmed histologically. He had a 2.5-cm epidermal inclusion cyst removed from the anterior neck, and at the same time he was noted to have a 2–3-cm firm swelling overlying the nape of the neck, which was not removed. Moreover, he was reported to have a number of firm skin lumps similar to those present in other family members.

The mother of the proband (II.2) has a 6 × 8-cm firm immobile lump overlying the insertion of the right erector spinae muscle in the posterior occipital region and present since childhood. In close proximity within the skull bone was a small osteoma, in addition to several mandibular osteomata visible on an orthopantomogram. She developed a palpable breast lump at the age of 33 years that had the mammographic appearance of a carcinoma. Histologically, this proved to be a desmoid tumor. A larger breast tumor removed several years later was also found to be a desmoid lesion. Colonoscopy at the age of 51 years revealed the presence of <50 scattered polyps most prevalent in the transverse and sigmoid colon. The two largest adenomas were 2–3 cm across, but most were ≤1 cm. Upper GI endoscopy on II.2 showed many small gastric fundus polyps but no duodenal or ampullary polyps. Her brother (II.4) was noted to have a rarefaction of the skull around the region of the torcula of uncertain significance. He also had two small (2 × 2 cm) firm deep lumps, one overlying the lower ribs and one on the arm. One brother was clinically unaffected (II.3), while the remaining brother (II.5) had a 4 × 5-cm firm tumor, again overlying the right occipital region, as well as several large swellings in the paraspinal muscles, the largest in the thoracic region causing a degree of scoliosis. Two tumors (from the forearm and the back) had been removed and histologically classified as desmoid tumors. Moreover, he developed a carcinoma of the head of the pancreas, which presented with jaundice and liver metastases. Duodenal or gastric polyps were not seen at upper GI endoscopy. He died within 6 mo. Lower GI endoscopy was not carried out. His two daughters were clinically affected, one (III.7) with a breast desmoid tumor and the second (III.6) with a small skin desmoid, a sebaceous cyst, and a large intraabdominal desmoid arising at 30 years of

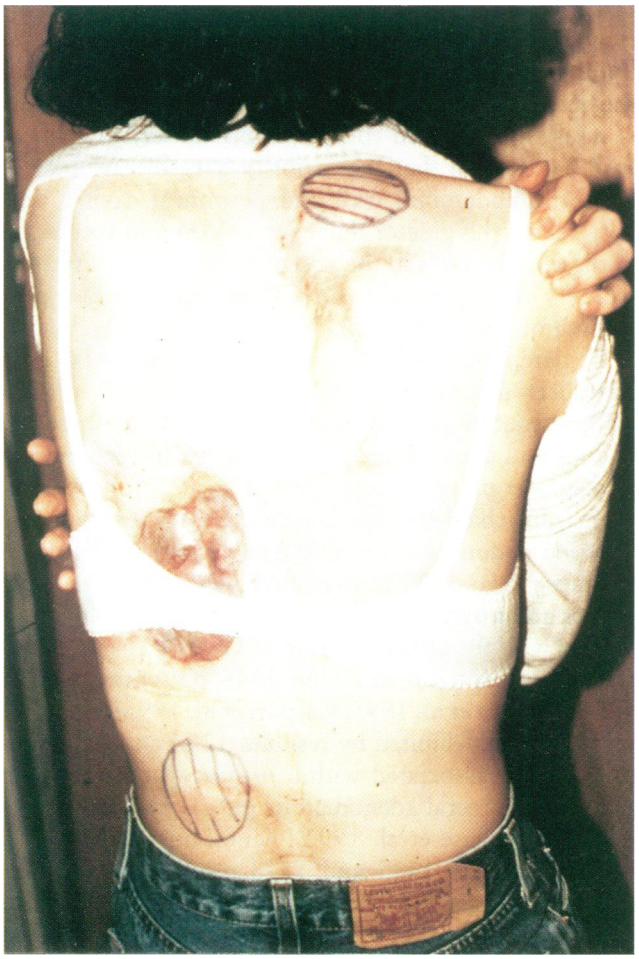

Figure 2 Appearance of the paraspinal desmoid disease of index case III.2.

age, which was successfully resected. She has recently developed a large and painful paraspinal desmoid tumor.

Examination of the colon in the 3d and 4th decades of life in II.4, III.1, III.2, III.3, III.6, and III.7 revealed no evidence of polyposis. Upper GI endoscopy was similarly normal, with the exception of a few sessile gastric polyps in II.2, II.4, and III.7. Fundoscopy in II.2 showed no evidence of CHRPE. Histological review of all the resected desmoid tumors confirmed them to be fibrous lesions composed of bland spindle cells with an infiltrating growth pattern through fat.

Cytogenetic Analysis

Karyotypic analysis of the intraabdominal desmoid from individual III.2 showed a cytogenetic deletion of chromosome 5q13-q22.3 in all 45 metaphases examined (fig. 3). In 30/45 tumor cells, this was the sole karyotypic abnormality, thus indicating that the 5q deletion had occurred at an early stage of tumor development. Peripheral white blood cells and skin fibroblasts karyotypes were normal.

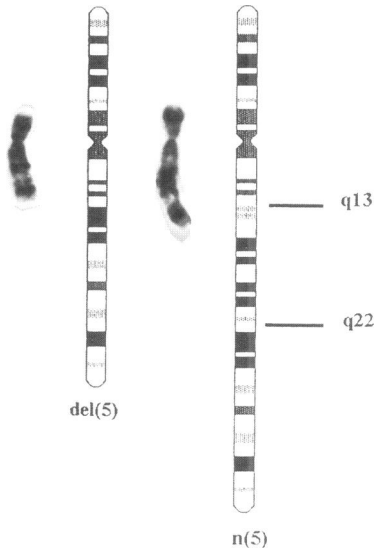

Figure 3 Interstitial deletion of chromosome 5q present in each of 45 metaphases examined from three separate regions of the intraabdominal desmoid tumor from III.2.

Linkage, Mutation, and Protein Analysis

Linkage analysis using markers flanking the *APC* locus on chromosome 5q21-q22 was entirely consistent with cosegregation of the disease with the *APC* gene (fig. 1). PTT analysis (Powell et al. 1993; Roest et al. 1993; van der Luijt et al. 1994) of the 3′ half of exon 15 demonstrated the presence of an early-termination codon between amino acids 1595 and 2344 (fig. 4). Sequence analysis of the corresponding exonic fragment revealed a 2-bp (+AA) insertion at codon 1924 leading to a frameshift and a stop codon 45 amino acids down-

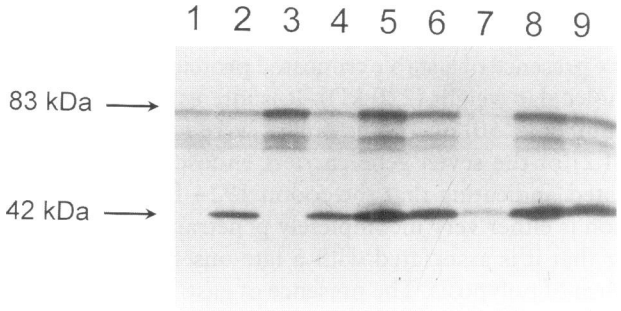

Figure 4 PTT analysis of individuals from the familial desmoid kindred depicted in figure 1. The 83-kD band represents the wild-type APC allele between codons 1595 and 2344. Samples from affected individuals show an additional 42-kD truncated polypeptide, indicative of the presence of a premature-termination codon. Lane 1, II.1. Lane 2, II.2. Lane 3, II.3. Lane 4, II.4. Lane 5, II.5. Lane 6, III.1. Lane 7, III.2. Lane 8, III.6. Lane 9, III.7.

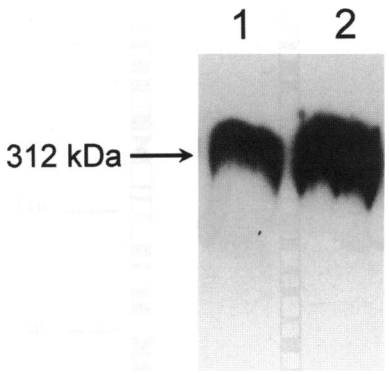

Figure 5 Western analysis of a fibroblast cell line from individual III.2 (lane 1) and of a control fibroblast cell line (lane 2). The 312-kD band corresponding to the *APC* full-length protein is indicated by an arrow.

stream. The mutation was demonstrated in all affected family members and was not present in unaffected individuals (data not shown). Moreover, PTT analysis of an additional desmoid tumor from individual III.2 clearly indicated the somatic loss of the wild-type allele (not shown).

Western blot analysis of both a fibroblast and a lymphoblastoid cell line derived from individual III.2 failed to indicate the presence of a truncated *APC* polypeptide of the expected length (220 kD, as predicted from the location of the chain-terminating mutation) (fig. 5). The latter result was confirmed by using two different antibodies, both raised against the NH_2-terminus of *APC*.

Discussion

The data presented here are consistent with the autosomal dominant desmoid disease observed in this kindred being caused by the *APC* germ-line mutation at codon 1924. Western analysis of a fibroblast cell line derived from an affected family member did not indicate the presence of a stable truncated protein of the expected molecular weight (220 kD). It is interesting that a small number (<50) of colonic polyps were found in only one (II.2) of the seven gene carriers endoscopically investigated, indicating that the codon 1924 frameshift mutation is either very incompletely penetrant in the GI tract or that it is associated with a late-onset and attenuated form of polyposis. The presence of gastric fundus polyps in three of the mutation carriers is in keeping with the so-called attenuated adenomatous polyposis coli, or AAPC. However, the penetrance of the *APC* codon 1924 mutation is 100%, as far as the desmoid disease is concerned. The complete penetrance of desmoid disease is of great interest, in view of the fact that infiltrative fibromatosis (desmoid tumors) and other extracolonic manifestations of FAP very rarely, if ever, affect every individual carrying the disease-causing *APC* mutation within a given kindred (Giardiello et al. 1994). Moreover, the site of the desmoid tumors arising in the HDD family members is unusual compared to the pattern observed in most FAP patients, in that the majority have disease remote from the abdominal wall and peritoneal cavity. This may reflect the greater tendency to desmoid formation in these individuals in the absence of abdominal trauma (surgery or pregnancy), which is often thought to trigger growth of desmoid tumors in FAP.

To date, >450 germ-line mutations of the *APC* gene have been described in FAP kindreds. More than 95% of these mutations lead to premature chain-terminating codons and truncation of the 312-kD wild-type protein into shorter polypeptides (Smith et al. 1993). In general, premature chain-terminating mutations approximately between codon 300 (exon 9) and codon 1600 lead to the more "classical" form of FAP with >1,000 colonic polyps and a variable incidence of extracolonic manifestations with the only exception of CHRPE that are consistently present up to codon 1444 (Olschwang et al. 1993; Bunyan et al. 1995; Caspari et al. 1995). A cluster of mutations delimited by residues 1250 and 1464 was found to be associated with a *profuse* phenotype with ≤5,000 colorectal adenomatous polyps per affected individual (Nagase et al. 1992). Mutations in the region between codons 1445 and 1578 result in severe desmoids, though never with 100% penetrance, but also osteomas, epidermoid cysts, and polyps of the upper GI tract, without CHRPE (Caspari et al. 1995). Spirio et al. (1993) have shown that chain-terminating mutations located at the 5′ end of the *APC* gene result in a milder FAP phenotype, AAPC, characterized by fewer polyps (<100) and a delayed age at onset. These early *APC* mutations are predicted to result in extremely short and presumably unstable truncated polypeptides. Germ-line mutations located downstream of codon 1600 are extremely rare and seem to result in a lower colorectal tumor multiplicity and variable extra-colonic phenotypes (Scott et al. 1995; Friedl et al. 1996; van der Luijt et al., in press). Western analysis revealed that, also in these cases, no stable truncated protein is present (van der Luijt et al., in press). Such phenotypic differences among FAP patients indicate that the length and stability of the shortened proteins are important determinants in establishing the severity of the corresponding tumor spectrum.

The 2-bp insertion at codon 1924 responsible for the desmoid disease in the present study is predicted to result in a truncated protein not detected by conventional Western analysis, at least in the lymphoblastoid- and fibroblast-derived cell lines investigated here. This could be due to instability of either the corresponding mRNA or of the truncated polypetide. Although the absence of

a stable truncated *APC* protein can explain the attenuated and late-onset polyposis phenotype, it cannot be the sole cause of the severe and completely penetrant desmoid disease as observed here. One possibility is that the stability of the mRNA and/or truncated protein is tissue dependent, so that the putative dominant-negative mode of action of the shorter polypeptide can only be exerted in a subset of tissues. Alternatively, one could postulate that the truncated protein is present but below the detection limit of the Western protocol employed here. In both cases, the predicted 220-kD truncated protein, approximately two-thirds of the full-length 312-kD *APC* protein, would not include the EB1-, DLG-, and microtubulin-binding domains, although it would retain a significant portion of the wild-type protein (codons 1500–1900) normally absent in FAP-associated truncated *APC* polypeptides. This region is known to contain three of the seven 20–amino acid repeats localized between codons 1262 and 2033 and thought to be partially responsible for the binding to β-catenin together with the three "major" 15–amino acid repeats located between codons 1000 and 1200 (Rubinfeld et al. 1993; Su et al. 1993*b*; Polakis 1995).

A second possibility is represented by the presence of a "desmoid" modifier gene closely linked *in cis* to the codon 1924 *APC* mutation. Modifier genes in general are thought to be responsible for the intrafamilial phenotypic variability observed among FAP-affected individuals (Giardiello et al. 1994). However, the putative modifier genes identified to date in mouse models are not syntenic with loci on chromosome 5 in man (Moen et al. 1992; Dietrich et al. 1993); therefore, phenotypic modification in all family members would require cosegregation of the disease-causing mutation with the same allele of the modifier gene. Taking into consideration that other unrelated kindreds have been described to carry 3′ *APC* germ-line mutations resulting in AAPC but without HDD (Scott et al. 1995; Friedl et al. 1996; van der Luijt et al., in press), the presence of a "desmoid" modifier gene closely linked to *APC* in the present pedigree seems to be plausible.

Maher et al. (1992) also described a family characterized by infiltrative fibromatosis of the mesentery and breast (familial infiltrative fibromatosis; OMIM 135290) in two members of a kindred without colonic polyps, osteomas, sebaceous cysts, and CHRPEs. Three other family members died of bowel cancer without manifesting polyposis, suggesting an unusual form of hereditary nonpolyposis colorectal cancer (HNPCC). However, the present report and previous evidence of LOH and somatic mutations at the *APC* locus in desmoid tumors from FAP patients (Sen-Gupta et al. 1993; Miyaki et al. 1993), strongly suggest *APC* as the gene responsible for familial desmoid disease associated with a late age at onset and attenuated form of polyposis, which could easily be overlooked in such a family. In fact, more recent studies have demonstrated the presence of a 3′ *APC* mutation in the kindred affected by familial infiltrative fibromatosis (Scott et al., in press). Allocation of a distinct OMIM entry for HDD may be appropriate in view of the consistency of the clinical features in three generations. Moreover, it would seem preferable to use the term "hereditary desmoid disease," which uses the usual descriptive clinical term and avoids confusion with the other seven OMIM entries under "fibromatosis."

In conclusion, the data presented here show that a truncating mutation at the 3′ of the *APC* gene results in a disease other than FAP, namely, HDD. The late-onset and low-penetrant intestinal polyposis and the consistent occurrence of fibromatosis observed in this kindred are likely to be due to the association of this unusual mutation with a linked "desmoid" modifier.

Acknowledgments

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